IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	Conf. #: **6904**
Paul A. Krieg	)	
	)	Art Unit: **1643**
Serial No. **10/799,417**	)	
	)	Examiner: **Lynn Anne Bristol**
Filed: **March 12, 2004**	)	
	)	
For: **METHODS FOR MODULATING ANGIOGENESIS WITH APELIN COMPOSITIONS**	)	

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Paul A. Krieg, hereby declare that:

1. I am a currently employed as a Professor in the Department of Cell Biology and Anatomy at the University of Arizona College of Medicine.

2. I have over 25 years experience working in the field of molecular and cell biology and embryonic development.

3. I am the sole inventor for the above-referenced patent application.

4. I have reviewed the above-referenced patent application, the currently pending claims, and the Office Action mailed April 19, 2007.

5. In my opinion, the Examiner is incorrect in asserting that the specification is not enabling for methods of using an apelin inhibitor to inhibit angiogenesis in a biological sample.

6. Example 5 clearly shows that an apelin antisense oligonucleotide does in fact inhibit angiogenesis in the art-accepted angiogenesis model system of *Xenopus laevis* embryos. The data presented in Example 5 show that an apelin inhibitor (*i.e.*, apelin antisense molecule) specifically inhibits angiogenesis in an art-accepted model system.

7. The specification also provides enabling support for methods using an apelin antibody or fragment thereof to inhibit angiogenesis. As the specification notes, methods for making antibodies and analyzing their binding specificity are well known in the art.

8. Millipore Bioscience Division produced a library of antibodies and screened these antibodies by ELISA assays to identify antibodies that potentially bind apelin. From these, I have identified two antibodies that specifically block apelin activity (ab208 and ab210). *See* Exhibit A. For these experiments, approximately 1 x 10^4 bEnd.3 cells were seeded into each well of an 8 well culture slide in DMEM high glucose medium containing 10% serum. Cells were allowed to attach overnight to the slide, and then the media were replaced with serum free media. After 24 hours of serum starvation, the media was replaced once again, this time with media containing 1 ng/ml apelin (0.67 nM) alone or together with 1 mg/ml anti-apelin antibody (9.0 nM). This represents approximately 15-fold molar excess of antibody. Four different anti-apelin antibody preparations named ab206, ab207, ab208, and ab210 were tested. The bEnd.3 endothelial cells were allowed to grow under these conditions for 24 hours. BrdU (10 mM) was added to each well for the final 2 hours of the culture, and then cells incorporating BrdU (*i.e.*, proliferating cells) were detected using standard methods. The reduction in proliferation numbers in the presence of ab208 and ab210 is statistically different from apelin stimulated numbers using Student's T test $P < 0.05$.

9. Moreover, others have identified additional antibodies that specifically bind apelin. *See, e.g.*, Kleinz and Davenport, 2004, Regulatory Peptides 118:119-25.

10. Further, the specification provides additional methods for determining that an antibody or other modulator specifically affects apelin activity and angiogenesis. For example, the effect of the antibody or other modulator may be determined in a chicken chorioallantoic membrane (CAM) assay by measuring vascular branching in the presence or absence of the antibody or other modulator.

11. I have identified an apelin antibody (ab208) that specifically inhibits angiogenesis in another art-accepted angiogenesis model, the CAM assay. In these experiments, 10 day old chicken chorioallantoic membranes (CAMs) were used for the angiogenesis experiment. Twelve CAMs were treated for each group at the start of the experiment. Negative control CAMs were treated with PBS. Positive control CAMs were treated with one application

of 10 ng apelin on a 3MM filter paper disk (*i.e.*, 10 ng total protein, not a concentration). Total amount is equivalent to about 6.7 pMols. The apelin + antibody experimental CAMs were treated with one application of 10 ng apelin plus 5 micrograms of Ab203 protein on a 3MM filter paper disk (*i.e.*, total amounts, not concentrations). Total antibody was equivalent to about 45 pMols. After 72 hours of incubation, CAMs are harvested. Any CAMs exhibiting hemorrhaging or where embryo died were excluded at this point. Angiogenesis was assayed by scoring of blood vessel branches. *See* Exhibit B. Overall, this experiment indicates that ab208 blocks apelin-induced angiogenesis. The reduction of vascular branching seen in the CAMs treated with apelin plus antibody is statistically different from that seen in apelin-induced CAMs using Students T-test, $P < 0.05$. The results clearly demonstrate that the specification is enabling for methods of using apelin antibody to inhibit angiogenesis in a biological sample.

11. I declare that all statements made herein of my own knowledge and belief are true and that all statements made on information and belief are believed to be true, and further that the statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Paul A. Krieg

Paul A. Krieg, Ph.D.

Sep 18, 2007

Date

Exhibit A

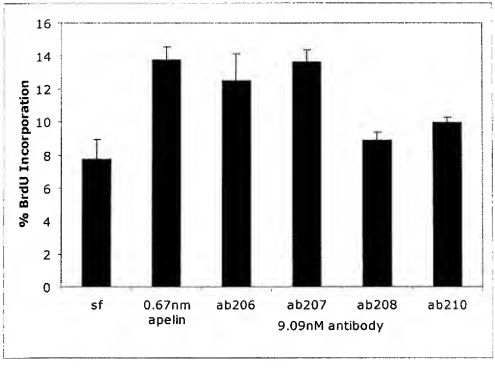
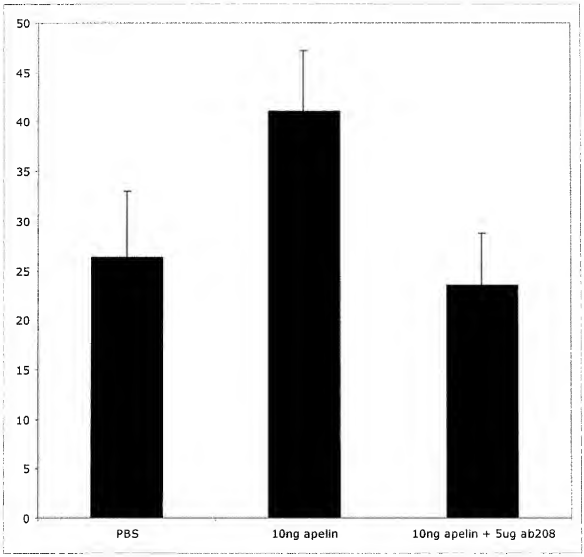

16
14
12
10
8
6
4
2
0
% BrdU Incorporation
sf
0.67nm apelin
ab206
ab207
ab208
ab210
9.09nM antibody

Exhibit B


50
45
40
35
30
25
20
15
10
5
0
PBS
10ng apelin
10ng apelin + 5ug ab208